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An assessment of acid wash and bioleaching pre-treating options to remove mercury from coal

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Abstract

The United States Environmental Protection Agency is expected to begin regulating the release of vapor-phase mercury from coal-fired power plants in the year 2007. Chemical pre-treatment methods were investigated for mercury removal effectiveness from pulverized low-sulfur North Dakota lignite coal. More limited results were obtained for a pulverized high-sulfur Blacksville bituminous coal. A two-step acid wash treatment showed removal rates of 60–90%, compared to one-step treatments with concentrated HCl, which yielded removals of 30–38%. Removal effectiveness is similar for first step solvents of water, pH 5.0 acid, or pH 2.0 acid followed by concentrated HCl as the second step solvent, and is independent of first step incubation time. Neither of two bacterial strains, *Thiobacillus ferrooxidans* and *T. thiooxidans*, was found effective for mercury removal.

Keywords: Coal; Acid wash; Mercury

1. Introduction

The United States Environmental Protection Agency (EPA) is expected to begin regulating the release of vaporphase mercury from coal-fired power plants in the year 2007. Therefore, the mitigation of mercury emissions from pulverized coal combustion is an active area of current research. For large-scale power plants, the use of end-of-the-pipe control technologies is expected to be justified [1]. For smaller scale users these end-of-the-pipe technologies may be economically prohibitive. An alternative to the end-of-pipe technology is to remove the pollutant of concern before combustion. Two possible techniques are removal by chemical pre-treatment (e.g. acid wash) and biological pre-treatment (e.g. bacteria).

Definitive determination of the forms of occurrence of mercury in coal is difficult. Leaching and float-sink studies have been used to infer associations of mercury in a wide variety of coals [2–4]. This association varies widely from coal to coal. However, the most likely forms of occurrence are association with epigenetic pyrite as HgS and as

organometallic compounds. Mercury has not typically been found to be associated with the silicate fractions of the inorganic material in coal.

Chemical pre-treatment has been found to be effective for specific pollutants, including sulfur [5–7]. Sulfur removals of greater then 80% have been reported [5]. Hydrogen peroxide in combination with sulfuric acid has been shown to remove up to 25% of the total sulfur from two Indian coals [7]. Steel and coworkers report success in removing sulfur from coal with a combination of hydrofluoric and nitric acids [6]. The use of a methanol/water mixture has given total sulfur removal up to 53% while the addition of potassium hydroxide to this water/methanol mixture increases the removal of sulfur up to 62% [5].

Unlike sulfur, the use of chemical coal cleaning techniques to remove mercury from coal has not been explored to a great extent. Chemical coal cleaning techniques with reported mercury removals of 41 and 58%, respectively, have been reported [8,9]. A wide variety of physical coal cleaning techniques has been explored for the removal of mercury from various coals. These techniques include cyclones, froth flotation, and selective agglomeration. The mercury removals vary widely with a typical maximum removal of around 60–70% [9]. Since

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EPA regulations are expected to require reductions much higher than 70%, current physical coal cleaning methods will probably not be adequate to meet the new regulations without additional control.

Biological resources have proven effective for the removal of trace amounts of toxic metals from waste streams [10–15]. Previous research has shown that many different biological resources (such as bacteria, fungi, plants, dead biomass, and algae) have the potential for mercury uptake [11,12]. Bacterial desulfurization of coal has been shown to remove anywhere from 10 to 90% sulfur depending on the type of coal and the type of bacteria [13–18].

The present study was conducted to evaluate pretreatment of coal for the removal of mercury by two techniques: chemical pre-treatment (more specifically by acid dissolution) and by bacteriological treatment. Concomitant removal of sulfur was also measured during these experiments.

2. Experimental methods

2.1. Coal characterization

Low-sulfur North Dakota lignite obtained from the Falkirk mine in North Dakota and high-sulfur Blacksville coal obtained from the Blacksville No. 2 mine in Pennsylvania were acquired from reference stocks maintained at the University of North Dakota Energy and Environmental Research Center (UND EERC). The coal was pulverized to a typical utility combustion grind of $70{-}80\%$ minus 200 mesh (76 μm diameter) and analyzed for the chemical content at the UND EERC. The key chemical analysis of the two coals is presented in Table 1.

Table 1 Chemical analysis of the two study coals

Analysis	Falkirk lignite	Blacksville bituminous			
Proximate analysis (as received, wt%)					
Ash	8.67	7.71			
Volatile matter	35	37.8			
Moisture	27.7	1.2			
Fixed carbon	28.6	53.3			
Heating value (MJ/kg)	17.24	31.49			
Ultimate analysis (as received, wt%)					
Carbon	43.6	74.7			
Moisture	27.7	1.2			
Ash	8.67	7.71			
Hydrogen	2.42	4.62			
Nitrogen	1.12	2.09			
Sulfur	0.77	2.03			
Oxygen	15.7	7.65			
Elemental analysis (ppm	Elemental analysis (ppmw as received)				
Mercury	0.087 ± 0.007	0.0905 ± 0.0045			

2.2. Acid wash

Both one-step and two-step acid wash protocols were investigated. The one-step acid wash protocol consisted of exposing the coal to one of four treatment solvents:

- (1) Neutral solution, pH 7.0 Ultrapure water
- (2) Mild acid at pH 5.0,
- (3) Moderate acid at pH 2.0
- (4) Harsh acid (concentrated HCl, 35 w/w%)

Incubation time for the coal in the solvent was varied from 45 min incubation time up to as much as 116 days. In addition, the harsh acid solvent experiments were run at two different solvent temperatures: 25 and 80 °C. The mild and moderate acid solvent cases employed the sterile culture media that was used for the bioleaching experiments (Section 2.3).

For the two-step treatment protocol, water, mild acid, or moderate acid was used as the first step solvent. First step solvent incubation was varied from 24 h incubation time up to as much as 116 days. The second step was treatment with concentrated HCl (harsh acid) at either 25 or 80 °C for 45 min.

For both one-step and two-step protocols, 40 g of dry coal were placed into a 2-l Erlenmeyer flask and solvent was added to a pulp density of 4 wt%. Samples were extracted via a pipette onto a Millipore Durapore PVDF 0.45 μm membrane filter where the liquid was removed by use of a filter flask and vacuum pump.

For the two-step protocol, the coal was then treated in the second step with concentrated HCl. For the hot acid tests, the coal was placed in a beaker of 30 ml hot, concentrated HCl (80 °C) for 20 min. The excess acid was removed using the filter flask and vacuum pump as described above. Adding the time required for filtration, the combined average incubation time for coal in hot HCl was 45 min. An additional 20 ml of hot HCl was used to rinse the remaining coal from the beaker. For the 25 °C acid tests the coal was rinsed with 50 ml of concentrated HCl and filtered to remove the excess acid.

Using these extraction protocols, coal samples of approximately 5 g were obtained. These were divided into three samples which were then analyzed for mercury concentration, moisture content, and sulfur concentration, respectively.

When ultrapure water was used in the first wash step, mixing occurred on the rotary shaker table described in Section 2.3 (below), at 180 rpm for 24 h. For mild and moderate acid solvent experiments, mixing and incubation was accomplished as described in Section 2.3, below. These samples served as the control samples for the bioleaching experiments.

2.3. Bioleaching

The pot scale bioleaching experiments were conducted in three main steps. First, large volumes of bacterial suspensions were grown for coal inoculation. The bacterial cultures used were *Thiobacillus ferrooxidans* (ATCC # 19859) and *T. thiooxidans* (ATCC # 15494). The *T. ferrooxidans* were chosen for their ability to live in acidic conditions (pH 2.0) and their utilization of Fe⁺² as an energy source [19]. The *T. thiooxidans* were chosen for their ability to live in mildly acidic conditions (pH 5.0) and their direct utilization of sulfur as an energy source [20].

The culture medium used for the *T. ferrooxidans* was a modified 9 K growth medium. It consisted of 3.0 g of (NH₄)₂SO₄, 0.1 g of KCl, 0.5 g of K₂HPO₄, 0.5 g of MgSO₄·7H₂O, 0.01 g of Ca(NO₃)₂, 44.8 g of FeSO₄·7 H₂O per liter of distilled water [19]. The pH of the culture medium was adjusted to 2.0 using concentrated sulfuric acid. The culture medium was prepared and placed in a pressure sterilizer at 20 psi for 30 min. This bacterial strain was cultured to a large volume (~13 l) by growth at 25 °C on a rotary shaker table at 180 rpm for 4 days.

The growth was monitored by optical density at 600 nm (OD_{600}) . Bacteria harvesting was conducted when OD_{600} reached 2.0. Once the *T. ferrooxidans* were cultured to a large volume, they were centrifuged at 7000 rpm for 20 min using an IEC HN-SII centrifuge. The bacteria pellet obtained was re-suspended by addition to 500 ml of fresh culture medium for use in inoculating the coal samples.

The culture medium used for the *T. thiooxidans* was ATCC medium # 125 [20]. It consisted of 0.2 g of (NH₄)₂SO₄, 0.5 g of MgSO₄·7H₂O, 0.25 g of CaCl₂, 3.0 g of KH₂PO₄, and 5.0 mg of FeSO₄ per liter of tap water. One gram of precipitated sulfur was placed in a dry, sterile flask. To this flask 100 ml of the culture medium was added so that the sulfur would float on the surface of the medium. The pH of the culture medium was adjusted to 5.0 using concentrated HCl. The culture medium was then sterilized in flowing steam for 30 min a day for three consecutive days.

The *T. thiooxidans* bacterial strain was cultured to a 200 ml volume at 25 °C for 20 days. These bacteria were placed in a stationary incubator at 25 °C. The growth was monitored by the precipitation of the sulfur as recommended by ATCC [21]. Once a visibly detectable sulfur precipitation occurred (after three weeks of growth) the *T. thiooxidans* were used directly, without centrifugation, to inoculate the coal samples.

The second step in the bioleaching protocol was to inoculate the coal samples with bacteria by placing 40 g of dry coal into a 2-1 Erlenmeyer flask, and adding 11 of a sterile culture medium to a 4% pulp density. Then a 10 ml inoculum of the concentrated bacteria ($OD_{600} = 2$) was added in the case of the *T. ferrooxidans*. A 10 ml inoculum was used directly from the cultured *T. thiooxidans*. Two flasks were left uninoculated to serve as control samples.

The control samples consisted of a 4% pulp density of coal and sterile culture media.

The flasks were then placed on a rotary shaker table at 180 rpm and incubated for times that varied from 3 days up to 116 days. The bioleaching was carried out over this extended period in order to insure that the *T. thiooxidans* had adequate time for growth.

The bioleaching coal samples were extracted and treated following the techniques described in Section 2.2. The samples were analyzed as described in Section 2.4.

2.4. Analytical techniques

The moisture content was determined as a weight difference upon drying in a 105 °C oven for 2 h. The moisture content was used to normalize the sulfur and mercury results to a dry coal basis.

The sulfur content was determined at the UND EERC's coal lab using a Leco SC-132 Sulfur Analyzer and ASTM method D 5016 [22]. The mercury content was determined by digesting the coal with a mixture of HCl and HNO₃ following the alternative digestion method for ASTM method D 3684 [22] in order to obtain a liquid sample for the analysis. After digestion the mercury content of the liquid was determined at the UND EERC analytical chemistry lab using a Cetac Technologies, Inc. Model M-6000A cold vapor atomic absorption spectrophotometry mercury analyzer and ASTM method D 6414 [23].

3. Results and discussion

3.1. Mercury removal from ND lignite by acid wash

Coal samples in both the one-step and two-step acid wash techniques were incubated for a variety of time periods (from 45 min to 116 days) in order to determine the effect of this parameter on mercury and sulfur removal. The results indicated that incubation time did not produce statistically different removals of mercury or sulfur and therefore incubation time was ignored as a variable in subsequent data analysis.

Mercury removal results for both one-step and two-step acid wash protocols are presented in Table 2. The one-step acid wash protocol provided mercury removals of 30–40%. Using HCl at 80 °C (hot) instead of at 25 °C (cold) did not result in any additional mercury removal.

By contrast, the two-step acid wash protocol provided mercury removals of 60–90%. A comparison of the pooled variances of results for all three first step solvents (pure water, mild pH 5.0 acid, and moderate pH 2.0 acid) yields a statistically insignificant difference between the three conditions at a 95% confidence interval. However, a comparison of the two-step and one-step protocol results reveals a significant improvement in mercury removal for

Table 2
Mercury removal from North Dakota lignite using one and two step acid wash protocols

Acid wash results	Average Hg removal (%)	Standard deviation	No of sample
One-step mild acid wash using culture medium at pH 5.0	35	9	5
One-step moderate acid wash using culture medium at pH 2.0	37	9	7
One-step harsh acid wash with 25 °C (cold) concentrated HCl	38	4	4
One-step harsh acid wash with 80 °C (hot) concentrated HCl	31	5	4
Two-step acid wash using neutral pre-treatment in ultrapure water followed by harsh acid wash at 25 °C (cold) with concentrated HCl	39	14	4
Two-step acid wash using neutral pre-treatment in ultrapure water followed by harsh acid wash at 80 °C (hot) with concen- trated HCl	77	13	4
Two-step acid wash using mild pre-treatment at pH 5.0 followed by harsh acid wash at 25 °C (cold) with concentrated HCl	44	9	3
Two-step acid wash using mild pre-treatment at pH 5.0 followed by harsh acid wash at 80 °C (hot) with concentrated HCl	57	10	4
Two-step acid wash using moderate pre-treatment at pH 2.0 followed by harsh acid wash at 25 °C (cold) with concentrated HCl	53	4	3
Two-step acid wash using moderate pre-treatment at pH 2.0 followed by harsh acid wash at 80 °C (hot) with concentrated HCl	69	19	4

the two-step hot acid wash protocol over the comparable one-step hot acid wash protocol. The two-step cold acid wash protocols also result in an increase in mercury removal compared to the comparable one-step acid treatment. However, the improvement is not as significant as that seen in the hot acid treatment.

Based on the results presented in Table 2, the mercury species in this lignite can be divided into two categories, those that are loosely bound and easily removed and those that are more tightly bound and require more aggressive techniques. The former can be easily removed, even by the very mild one-step (pH 5.0) acid wash treatment, whereas

the latter can only be partially removed even by using a harsh high-temperature concentrated acid treatment. However, these more tightly bound mercury compounds are more effectively dissolved when an initial wetting agent is applied to the lignite prior to treatment with concentrated HCl. This may be due to swelling of coal upon its long-term contact (i.e. 24 h or greater) with an aqueous medium resulting in a greater accessibility of the mercury-containing sites. The pH of this initial wetting agent does not appear to be important since using pH 7.0 water as the first step solvent appears to yield comparable mercury removal results as using pH 5.0 or 2.0 acids.

3.2. Mercury removal from ND lignite by bioleaching

The mercury removal results of the bioleaching experiments are presented in Table 3. Comparison of the first two rows, which show Hg removal from exposure to bacteria in the culture solution, to the last two rows, which show Hg removal from exposure to the culture solutions without any bacteria, indicates a slightly higher mercury removal for the bioleached coal samples compared to the control samples. However, this difference is not statistically significant. The control experiments are essentially the same as the one-step acid wash in pH 5.0 and 2.0 culture solutions whose data are reported in Table 2, respectively. These results lead to the conclusion that the mercury removal observed is most likely due to the solvent effect of the acidic culture media rather than any metabolic reaction by the bacteria. Therefore, the bioleaching technique does not appear to be effective for the removal of mercury from this lignite coal.

3.3. Mercury removal from Blacksville coal by acid wash

A more limited set of results was obtained for the Blacksville coal. These results are presented in Table 4. The treatment of Blacksville coal with the hot concentrated HCl yielded mercury removal of greater then 60%. However, the results indicate that an initial wetting step by water is not effective for improving mercury removal over a single concentrated HCl wash. Future work is necessary to

Table 3
Mercury removal from North Dakota lignite by bioleaching

Bioleaching results	Average Hg removal (%)	Standard deviation	No of samples
T. ferrooxidans	42	8	4
T. thiooxidans	38	10	2
T. ferrooxidans control ^a	31	8	3
T. thiooxidans control ^a	32	9	3

^a Leaching in the culture medium without the presence of bacteria.

Table 4
Mercury removal from Blacksville coal using an acid wash chemical treatment

Acid wash results	Average Hg removal (%)	Standard deviation	No of samples
Two-step acid wash using neutral pre-treatment in ultra- pure water followed by harsh acid wash with 80 °C (hot) concentrated HCl	41	18	4
One-step harsh acid wash with 80 °C (hot) concentrated HCl	65	4	4

determine if an acid wetting agent in the first step provides more attractive removal results.

Comparison of the results for comparable treatment protocols in Tables 2 and 4 reveals that similar mercury removal was achieved with two very different coals. This suggests that the acid wash technique for the removal of mercury is not specific to lignite coal but may be generally applicable to a wide range of coals. Future work is necessary to verify the generality of these results.

3.4. Sulfur removal by acid wash

Sulfur removal effectiveness was also measured during the acid wash experiments described in Sections 3.1 and 3.2, above. These results for experiments involving the North Dakota lignite are presented in Table 5. The one-step acid wash gave sulfur removals of 15–19% independent of the solvent used.

The two-step acid treatment protocols generated sulfur removals of up to 90%. Choice of first step solvent had a substantial effect on the removal efficiencies achieved and was directly related to the strength of the acid used in this step. This indicates that the first step acidic solvent plays a role in disassociating bound sulfur from the coal matrix, allowing for removal during the second step of the treatment protocol. As with the mercury results, first step incubation time (varied over the range of 24 h to 116 days) had no effect on the sulfur removal achieved.

Consistent with the mercury results, the temperature of the second step acid wash impacts the removal of sulfur from the coal. Hot HCl generated higher sulfur removal than cold HCl for both mild and moderate first step solvent experiments.

For the Blacksville coal, the difference between the one and two-step acid wash techniques is not statistically significant in the limited set of results obtained. Sulfur removals of 20-30% were obtained for the two cases investigated: one-step treatment with hot HCl and two-step treatment with a pure water first step solvent and hot HCl second step solvent.

Table 5
Sulfur removal from North Dakota lignite using one and two step acid wash protocols

Acid wash results	Average S removal (%)	Standard deviation	No of samples
One-step mild acid wash at pH 5.0	15	7	8
One-step moderate acid wash at pH 2.0	18	6	6
One-step harsh acid wash with concentrated HCl	19	N/A	1
Two-step acid wash using mild pre-treatment at pH 5.0 followed by harsh acid wash at 25 °C (cold) with concentrated HCl	14	1	2
Two-step acid wash using mild pre-treatment at pH 5.0 followed by harsh acid wash at 80 °C (hot) with concentrated HCl	37	6	2
Two-step acid wash using moderate pre-treatment at pH 2.0 followed by harsh acid wash at 25 °C (cold) with concentrated HCl	81	12	3
Two-step acid wash using moderate pre-treatment at pH 2.0 followed by harsh acid wash at 80 °C (hot) with concentrated HCl	91	1	3

4. Conclusions

Chemical pre-treatment methods were investigated for mercury removal effectiveness from low-sulfur North Dakota lignite coal. A two-step acid wash treatment yielded mercury removal rates of 60–90%. Removal effectiveness is similar for first step solvents of water, pH 5.0 acid, or pH 2.0 acid followed by concentrated HCl as the second step solvent, and is independent of first step incubation time. Using 80 °C HCl is more effective than 25 °C HCl during the second step. One-step treatment with concentrated HCl yielded mercury removals of 30–38%. More limited results obtained for a high-sulfur Blacksville bituminous coal were similar.

Significant concomitant sulfur removal was achieved using a two-step acid wash treatment involving pH 2.0 acid as the first step solvent followed by concentrated HCl (80–90%). For the one-step treatment protocols, sulfur removals of 15–19% were obtained.

It is interesting to note that under some conditions (i.e. two-step protocol using water as the first step solvent) significant amounts of mercury can be removed without similar concomitant sulfur removal. This uncoupling of mercury and sulfur removal indicates that the bulk of mercury may be present in the organic rather than inorganic

fraction of coal. Removal of up to 70–90% of mercury from ND lignite by the two-step acid wash protocol also indicates that mercury cannot be present in significant quantities in the inorganic pyrite fraction as mercury sulfide since HgS is insoluble in concentrated hydrochloric acid. This conclusion appears to be applicable to a high-sulfur Blacksville coal as well. However, the latter yielded a lower percentage of mercury removal so it may have more mercury present in a sulfide form not removable by acids.

Neither of two bacterial strains, *T. ferrooxidans* and *T. thiooxidans*, was found effective for mercury removal.

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References

- United States Environmental Protection Agency, Mercury study report to congress, vol. I. Executive summary. Office of Air Quality Planning and Standards and Office of Research and Development; December 1997. EPA-452/R-97-003.
- [2] Finkelman RB. In: Swaine DJ, Goodarzi F, editors. Modes of occurrence of environmentally sensitive trace elements in coal in environmental aspects of trace elements in coal. Boston: Kluwer Academic Publishers; 1995.
- [3] Senior CL, Bool III LE, Huggins F, Huffman GP, Shah N, Wendt JOL, Seames W, Peterson T, Shadman F, Helble J, Wornat M, Sarofim A, Olmez I, Crowley S, Finkelman R. Toxic substances from coal combustion—a comprehensive assessment. Phase I Final Report; September 1997 [DOE Contract DE-AC22-95PC95101].
- [4] Senior CL, Huggins F, Huffman GP, Shah N, Yap N, Wendt JOL, Seames WS, Ames MR, Sarofim AF, Swenson S, Lighty JS, Kolker A, Finkelman R, Palmer CA, Mroczkowski SJ, Helble JJ, Mamani-Paco R, Sterling R, Dunham G, Miller S. Toxic substances from coal

- combustion—a comprehensive assessment. Final Report; July 2001 [DOE Contract No. DE-AC22-95PC95101].
- [5] Ratanakandilok S, Ngamprasertsith S, Prasassarakich P. Coal desulfurization with methanol/water and methanol/KOH. Fuel 2001; 80:1937–42.
- [6] Steel KM, Patrick JW. The production of ultra clean coal by chemical demineralization. Fuel 2001;80:2019–23.
- [7] Mukherjee S, Mahiuddin S, Borthakur PC. Demineralization and desulfurization of subbituminous coal with hydrogen peroxide. Energy Fuels 2001;15:1418-24.
- [8] Center for Clean Air Policy, Mercury emissions from coal-fired power plants, science, technology and policy options; 1998.
- [9] Pavlish JH, Sondreal EA, Mann MD, Olson ES, Galbreath DL, Benson SA. Status review of mercury control options for coal-fired power plants. Fuel Process Technol 2003;82:89–165.
- [10] Brauckmann BM. Biosorption of heavy metals. Ann Arbor: CRC Press; 1990. p. 51-64.
- [11] Cantafio AW, Hagen KID, Lewis GE, Bledsoe TL, Nunan KM, Macy JM. Pilot-scale selenium bioremediation of San Joaquin drainage water with Thauera selenatis. Appl Environ Microbiol 1996;62:3298–304.
- [12] Brombacher C, Bachofen R, Brandl H. Development of a laboratoryscale leaching plant for metal extraction from fly ash by *Thiobacillus* strains. Appl Environ Microbiol 1998;64:1237–41.
- [13] Chen SY, Lin JG. Influence of solid content on bioleaching of heavy metals from contaminated sediment by *Thiobacillus* spp. J Chem Technol Biotechnol 2000;75:649–56.
- [14] Kozliak EI, Sternberg SR, Jacobson ML, Kuether KW, Mann MD. Mercury removal from air by a fiber-based bioreactor. Bioremediation J 1999;3:291–8.
- [15] Dobler IW, Canstein H, Li Y, Timmis KN, Deckwer WD. Removal of mercury from chemical wastewater by microorganisms in technical scale. Environ Sci Technol 2000;34:4628–34.
- [16] Acharya C, Kar RN, Sukla LB. Bacterial removal of sulphur from three different coals. Fuel 2001;80:2207-16.
- [17] Moran A, Cara J, Miles N, Shah C. Biodesulphurization of coal: behaviour of trace elements. Fuel 2002;81:299-304.
- [18] Rubiera F, Arenillas A, Martinez O, Moran A, Fuente E, Pis JJ. Biodesulfurization of coals of different rank: effect on combustion behavior. Environ Sci Technol 1999;33:476–81.
- [19] Harvey PI, Crundwell FK. Growth of *Thiobacillus ferrooxidans*: a novel experimental design for batch growth and bacterial leaching studies. Appl Environ Microbiol 1997;63:2586–92.
- [20] Tripathy SS, Kar RN, Mishra SK, Twardowska I, Sukla LB. Effect of chemical pretreatment on bacterial desulphurization of Assam coal. Fuel 1998;77:859–64.
- [21] American Type Culture Collection. Product information sheet for ATCC 15494. Manassas, VA.
- [22] American Society for Testing and Materials, Annual book of ASTM standards. Philadelphia, PA: ASTM; 1970.
- [23] American Society for Testing and Materials. Annual book of ASTM standards, vol. 5.06. Philadelphia, PA: ASTM;2002.